REMARKS

Claim 1 is pending and has been amended. New claims 26-29 have been added. No new matter has been introduced thereby.

Claim 26 is drawn to a variant of the method of claim 1, wherein mutagenic PCR is applied to mutating DNA encoding the sensor domain of a DmpR protein without necessarily first removing the sensor domain DNA from a DmpR DNA, followed by ligation of the resulting mutated sensor domain fragment into a DNA encoding the DmpR protein from which the corresponding sensor domain fragment has been removed. This method is specifically exemplified in the subject application, and support may be found at page 6, paragraph beginning at line 21, and at page 9, first two paragraphs.

Claim 27 is drawn to the method of claim 26, wherein the phenols and substituted phenols to which bacteria carrying a mutated DmpR sensor domain respond are selected from a specified group, which group corresponds to phenol and substituted phenols to which specific DmpR sensor domain mutants disclosed in the specification responded, generating enhanced transcriptional activation. Support for this claim may be found, inter alia, at page 11, line 4 through page 12, line 22, and FIGS. 2-7.

Claim 28 is drawn to the method of claim 26, wherein the specific PCR primers of SEQ ID NOS: 16 and 17 are used to drive the mutagenic PCR. Support for this claim may be found on page 9, lines 6 and 7.

Claim 29 is drawn to the method of claim 27, wherein the resulting transcriptional activation of the reporter gene is enhanced by at least 4-fold. Support for this claim may be found in the results presented in FIGS. 2-7 and discussed at pages 11 and 12, which establish that the method of the invention is capable of

producing a 4-fold or greater enhancement for responses to this group of phenols and substituted phenols.

REJECTIONS UNDER 35 USC 112, SECOND PARAGRAPH

Claim 1 was rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Firstly, the Examiner states that the limitation "the sensor domain" is unclear and lacks antecedent basis. Secondly, the Examiner states that claim 1 is confusing in the recitation of "removing the sensor domain from the DNA encoding the DmpR protein. In response, claim 1 has been amended essentially as the Examiner suggested in paragraphs 7 (a) and (b). Applicants therefore request reconsideration and withdrawal of the rejection.

REJECTION UNDER 35 USC 103

Claim 1 was rejected under 35 USC 103(a) as being unpatentable over Pavel et al. in view of Caldwell et al. The rejection has been carefully considered.

The Office's case for obviousness now appears to rest, essentially, on (1) the conclusion that a skilled artisan would recognize from Pavel that effector specificity mutants should be created by mutating *only* the sensor domain, and (2) the assumption that the particular method used by applicants for generating mutations would have been selected by one of ordinary skill in the art.

Firstly, with respect to the conclusion (1) above, at page 6, line 3, the Examiner states that *"Pavel teach that their objective was to identify an effector specificity mutant (page 7554, left column, middle), which resulted from mutation only within the sensor domain of DmpR..."* This statement appears to be factually incorrect,

as the referenced text does not indicate that these researchers were looking for sensor-domain specific mutants.

Moreover, Pavel et al. do not explain precisely how a mutation in the sensor domain results in the acquisition of an ability to recognize a novel effector compound. This is relevant, since Pavel's results would not necessarily lead one of ordinary skill in the art to *limit* mutations to the sensor domain in attempting to construct DmpR effector mutants. In fact, Pavel et al. specifically recognize that the genetic system used to monitor activation of the regulator *"do not discriminate between mutations that enhance putative effector binding and those which enhance the result of the presumed conformational change to an active form. To date nothing is known about the mechanism by which interaction of DmpR with aromatic effectors leads to activation."* (page 1556, left column, bottom to right column, top). Note the authors' use of the terms "putative" and "presumed", which underscores the fact that these authors concluded that their results, in combination with those from other labs, only *"suggest that activation of DmpR and XylR is mediated by aromatic effector binding to the A domains of these regulators"* (page 1556, left column, bottom).

Similarly, as noted in a publication by two of the inventors of this application (Wise and Kuske, 2000, Applied Environ. Microbiol. 66: 163-169), *"The mechanism by which DmpR binds its chemical effectors and changes conformation to become capable of transcriptional activation is not well understood. However, there is good evidence that the capacity of DmpR to activate transcription is repressed through an interaction between the sensor domain and polymerase-activating domains of DmpR"* (citing Ng et al., 1996, J. Biol. Chem. 271: 17281-86; O'Neil et al., 1998, Mol. Microbiol. 28: 131-141; and Shingler and Pavel, 1995, Mol. Microbiol. 17: 505-513). These authors also noted that *"It has been suggested that mutations that alter the effector profile of DmpR or XylR exert their effect either through an improvement in the effector-protein interaction or by changing the three-dimensional structure of the protein*

in ways that enhance other necessary functions of the protein, for example, polymerase activation" (citing Delgato and Ramos, 1994, J. Biol. Chem. 269: 8059-62; Pavel et al., 1994, J. Bacteriol. 176: 7550-7556; and Salto et al., 1998, J. Bacteriol. 180: 600-604).

Thus, from the perspective of one of ordinary skill in the art, the prior art (including, particularly, the cited Pavel et al. reference) certainly did not rule out the possibility that a mutation which changes the three-dimensional structure of DmpR could indeed enhance or expand the protein's effector profile, or the possibility that mutations outside of the sensor domain (with or without mutations in the sensor domain) could result in such three-dimensional changes. It is therefore not necessarily the case that one seeking to create DmpR effector mutants would have selected a mutational approach that eliminates the possibility of generating mutations outside of the sensor domain. Again, there is certainly no indication or suggestion in the cited Pavel et al. reference that one should *avoid* a mutational approach that targets other parts of the DmpR protein. Therefore, applicants respectfully suggest that the Office's conclusion that one would have been motivated to mutate *only* the sensor domain is in error.

Secondly, even if Pavel can be taken to suggest the desirability of mutating only the sensor domain, which is denied, there is clearly no suggestion in Pavel that the particular mutagenic technique employed by applicants should be selected from the many different mutagenesis techniques that were available to those skilled in the art at the time the invention was made. Indeed, as indicated in applicant's previous response (Paper No. 17), one of ordinary skill could well have interpreted the teachings of Pavel to suggest that chemical mutagenesis techniques may be productively employed to generate mutations. It is one thing to say that Pavel's effector specificity mutant had no mutations in the other parts of the DmpR gene, but quite another to say that Pavel *teaches* that effector specificity mutants must *only* contain mutations in the sensor domain. Pavel shows that the chemical mutagenesis technique worked to generate at least one

DmpR effector mutant. Thus, one of ordinary skill may well have been motivated to repeat Pavel's experiment in order to screen for additional effector specificity mutants. In the absence of evidence to the contrary, this possibility cannot be dismissed.

At the time the invention was made, there were numerous methods being used for introducing mutations into DNA, including but not limited to oligonucleotide-directed mutagenesis (e.g., Dalbadie-McFarland et al., 1982, Proc. Natl. Acad. Sci. USA 79: 6409-13), DNA shuffling (Stemmer and Crameri, WO 97/20078), transposon-mediated mutagenesis (e.g., Tomb et al., 1989, J. Bacteriol. 171: 3796-3802; Gwinn et al., 1997, J. Bacteriol. 179: 7315-20; Hutchinson et al., 1999, Science 286: 2165-69), error-prone PCR methods (e.g., Leung, 1989, Technique 1:11-15; Bartel and Szostak, 1993, Science 261: 1411-18), including the mutagenic PCR technique utilized by applicants (Cadwell and Joyce, 1992, PCR Methods and Applications 2: 28-33), cassette mutagenesis (e.g., Delgrave et al., 1993, Protein Engineering 6: 327-331; Arkin and Youvan, 1992, Proc. Natl. Acad. Sci. USA 89: 7811-15; Oliphant et al., 1986, Gene 44: 177-183; Hermes et al. 1990, proc. Natl. Acad. Sci. USA 87: 696-700), the use of mutator strains to add mutational frequency (Greener and Callahan, 1995, Strat. Mol. Biol. 7: 32), *in vivo* site specific recombination systems (e.g., Nissim et al., EMBO J. 13: 692-698; Winter et al., 1994, Ann. Rev. Immunol. 12: 433-455; Caren et al., 1994, Bio/Technology 12: 517-520; Hayashi et al., 1994, Biotechniques 17: 310-315; Calogero et al., 1992, FEMS Microbiology Lett. 97: 41-44; Galizzi et al., WO 91/01087; Radman et al., WO 90/07576), deletion and nested deletion strategies, and recursive sequence recombination (Minshull and Stemmer, USPN 5,837,458).

The mutagenic PCR method described in Cadwell et al., was just one of the various methods available at the time. However, the Office has not explained why one of ordinary skill in the art would have been drawn to this particular

method, when there were so many others to chose from, including those capable of generating a library of mutants.

Indeed, in the Office's previous iteration of this rejection (Paper No. 15), it was argued that the Willardson patent provided an example of (and motivation for) mutating a particular domain of a protein. Although this reference has been withdrawn from the rejection as presently formulated, it is important to note that Willardson used the mutational technique "recursive sequence recombination" to generate mutations. The question of why one of ordinary skill in the art would not have chosen this method, over the method of Cadwell, is certainly relevant but has not been addressed by the Office. In addition, Stemmer, another 103 reference used in prior Office Actions (Paper Nos. 4 and 15), teaches the use of gene shuffling to generate mutations. Again, the question of why one of ordinary skill would have been drawn to the use of mutagenic PCR, rather than gene shuffling, has not been addressed by the Office. In fact, in both the April 30, 2001 and August 13, 2002 Office Actions (Paper Nos. 4 and 15), the Office stated that Stemmer *"teach that one would use gene shuffling over mutagenic PCR because mutagenic PCR is not combinatorial and thus, is more limited in the number of possible mutations."* Thus, the Office has twice adopted the position that Stemmer teaches away from the use of mutagenic PCR. Accordingly, based on the Office's own reasoning, it seems quite unlikely that one of ordinary skill would have combined Pavel with Cadwell.

In view of the fact that there were numerous DNA mutagenesis techniques that one of ordinary skill could have applied to the generation of effector specificity mutants of the DmpR protein, it appears that the Office has engaged in hindsight reconstruction of the invention, concluding that one would limit mutations only to the sensor domain, and picking Cadwell from all of the other available mutagenesis techniques, because this was the method used by applicants. It is important to emphasize that the use of mutagenic PCR is a specific limitation of claim 1. Nowhere in any of the cited art is there a suggestion that Cadwell's

method should be applied to the problem the applicants aimed to solve. Only the applicants used the mutagenic PCR method for generating sensor domain point mutations. This particular selection, which only applicants made, would not have been the obvious choice to one of ordinary skill in the art.

The Office argues that because Pavel demonstrates that a point mutation in the sensor domain of the DmpR protein results in a changed effector specificity, one of skill in the art would have been motivated to generate other specificity mutants by limiting mutations to the sensor domain. However, the skilled artisan may instead have been motivated to simply create non-specific mutations and screen for effector specificity changes, particularly in view of the plain fact that Pavel was able to generate at least one effector specificity mutant using a non-specific chemical mutagenesis technique and a screening protocol. The Office has not explained why one would have been motivated to depart from the teachings of Pavel and adopt a different mutational paradigm. Moreover, even if one of skill would have been motivated to generate sensor-domain specific mutations, there were many different techniques that would have been available at the time of the invention. There is no suggestion in Pavel, whatsoever, that a mutational strategy aimed at relatively low-level single base point mutations would be desirable or should be considered, a fact that the Office has not denied in previous papers.

The Office has not provided any evidence to support the assumption that the specific technique successfully adopted by applicants would have also been adopted by a skilled artisan with no knowledge of the claimed invention. The explanation offered for why one of ordinary skill in the art would have been motivated to use the particular mutagenic technique adopted by applicants, mutagenic PCR, is conclusory. The Examiner states that *"one would have been motivated to use mutagenic PCR to mutate the DmpR sensor domain in order to avoid the use of a DNA mutagen as used by Pavel et al. and because of the teachings of Cadwell et al. who taught that by using mutagenic PCR, one can*

create a library of mutants by random mutagenesis, thus increasing the number of potential mutations." Thus, the Examiner's reasoning for concluding that one would have been motivated to use mutagenic PCR rests on the apparent assumption that one would have wanted to "avoid the use of a DNA mutagen" and the assumption that the teachings of Cadwell somehow also would have provided additional motivation. These assumptions are not supported by any evidence, and therefore this reasoning is conclusory. In particular, what evidence is there that one of skill in the art would have wanted to "avoid the use of a DNA mutagen"? The fact is, Pavel et al. used a chemical DNA mutagen, and these authors do not in any way suggest that the use of a chemical DNA mutagen is somehow undesirable, let alone suggest that one may utilize another mutagenic technique more productively. Accordingly, the rejection does not adequately address the issue of motivation to combine these two references.

The question of motivation to make the specific combination of elements that applicants made to produce the invention is a factual question which is clearly material to patentability under 35 USC 103. *MPEP 2143.01; In re Lee 277 F. 3d 1338, 1344 (Fed. Cir. 2002). "[A] showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'." Brown & Williamson Tobacco Corp. v. Phillip Morris Inc., 229 F.3d 1120, 1124-25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000), quoting C.R. Bard Inc., v. M3 Systems, Inc., 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998).* Moreover, a conclusion that one of ordinary skill in the art would indeed have been motivated to combine teachings in different references must rest on *specific* evidence, and *"the need for specificity pervades"* the case law. *In re Lee 277 F. 3d 1338, 1343 (Fed. Cir. 2002).* Here, the Office has provided no specific evidence, only conclusory statements, and thus appears to have succumbed to the insidious hindsight trap. Thus, the Office has not met its obligation to provide a factual basis, supported by specific evidence, for the rejection.

In conclusion, even assuming, *arguendo*, that the conclusion (1), above, is a sound one, the Office's assumption that such a skilled artisan would have selected the particular method used by applicants and claimed in this application as the method for generating the mutations is unsupportable. In fact, given all of the various methods that such a skilled artisan could have selected, it is far more likely that one of the methods utilized in either Pavel, or the previously cited Stemmer or Minshull references, would have been adopted, due to the fact that these methods were being used by scientists working in related fields to deal with related problems.

Accordingly, the claimed method is not obvious. Applicants urge the Office to reconsider and withdraw the rejection in light of the above.

Respectfully submitted,

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